IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/019,164	Confirmation No. 3977
Applicant	:	Benjamin J. Metcalf	
Filed	:	December 20, 2001	
TC/A.U.	:	1645	
Examiner	:	Patricia Ann Duffy	
Docket No.	:	ACY33484-00	
Customer No.	:	25291	

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DECLARATION UNDER 37 CFR 1.132

Sir:

I, Susan K. Hoiseth, do hereby state and declare that:

1. I am a citizen of the United States, residing in Suffern, New York.

2. I am currently employed by Wyeth and have held the position of Associate Director of Wyeth Vaccines Research since 1999. I obtained a B.A. from Luther College in 1978 and a Ph.D. from Stanford University School of Medicine in 1983.

3. From 1993 to 1999, I managed the Molecular Biology and Genetics Department at Lederle-Praxis Biologics (now Wyeth Vaccines Research) where I provided administrative and scientific oversight for up to 13 scientists, working on eight different bacterial pathogens, including *H. influenzae*. I also established within the department and still oversee, a group to work on optimization of recombinant protein expression. I therefore have considerable experience in this field and consider myself to be very competent to provide the statements contained in this declaration. A copy of my CV is attached.

4. I have read and am familiar with the present patent application and the Office Action dated May 13, 2004. I am particularly familiar with the primary reference cited by the Examiner – Anilionis et al. (WO 90/02557) – as the subject matter of this patent application came out of the same laboratory, Praxis Biologics, which is now Wyeth Vaccines Research. As such, the scientists under my direction were well acquainted with the failure of Anilionis et al. to express lipidated P6 in large amounts, and they resolved the problem by constructing plasmids that contain tightly regulated promoters operatively linked to the genes that encode the lipidated proteins. It is this plasmid construct – the construct of the present invention – that produces increased levels of recombinant P6 protein expression.

5. In support of the present application, I define below "tightly" regulated promoters, "leaky" promoters, and "strong" promoters as those terms are understood by a person of at least ordinary skill in the art.

6. Regulated promoters direct transcription at varying levels depending on environmental conditions. For example, the concentrations of lactose (or lactose analogs) and glucose in the growth media affect the level of transcription from the *lac* promoter. Promoters such as lac are well known to one skilled in the art to be regulated, but leaky (Guzman, et al. *Journal of Bacteriology*, 177(14):4121-4130, 1995; Georgiou, G. "Expression of Proteins in Bacteria." In *Protein Engineering: Principles and Practice*, J.L. Cleland and C.S. Craik, eds. Wiley-Liss, Inc., NY, 1996). The patent specification also references the art in which other authors (e.g., Guzman 1995) define the lac promoter as leaky, and the arabinose promoter as tightly regulated. Specifically, a leaky promoter induces some level of protein expression in the absence of any induction (Georgiou, p. 105). Page 3 of the present specification (lines 29 – 35) provides trc, tac, lac and P_L–C1857 as examples of promoters that are "not under tight transcriptional regulation," and refers to their "somewhat leaky transcription."

7. A tightly regulated promoter, on the other hand, is the opposite of a leaky promoter. Unlike a leaky promoter that is always partially "on" even in the uninduced state, a tightly regulated promoter refers to the degree to which a promoter can be maintained in the "off" state in the absence of induction. One can think of a leaky promoter as a very leaky faucet that cannot be shut off, and a tight promoter as a faucet

that is shut off or has only a very minor leak. It is a matter of degree. In one embodiment of the present invention, described on Page 8 (lines 27-32) of the specification, the plasmid (pPX4020) was constructed to contain the arabinose inducible promoter because this promoter "is tightly regulated and almost completely inactive if no arabinose is present and some glucose is present." Guzman also refers to the arabinose promoter as a tightly regulated promoter.

8. A "tightly" regulated promoter is not to be confused with a "strong" promoter. Strength refers to the maximum amount of transcription that can be achieved in the fully induced, or "on" state. A "strong" promoter would therefore be used to obtain a high level of transcription. Georgiou (pg. 104) refers to strong promoters as "promoters that give very high rates of transcription initiation." A "strong" promoter can also be a leaky promoter, and a tightly regulated promoter is not necessarily a strong promoter. Furthermore, a stronger promoter is not always better (Georgiou, 1996).

9. Anilionis used strong promoters, such as the lac promoter, to obtain high levels of transcription, but Anilionis was unable to obtain with such promoters a high level of lipidated P6 expression. Example 8 (page 81, lines 23-25) of Anilionis explicitly states that "[w]hen PBOMP-1 [P6] was expressed from *lac* or P_L promoters in *E. coli* JM103 orHB101 strain, only low levels of PBOMP-1 were expressed." Therefore, the present invention solves a problem that Anilionis was unable to solve.

The undersigned declarant declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing from the present application.

Signed this 11th day of November, 2004

Susan K. Hoiseth

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CURRICULUM VITAE

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POSITIONS HELD:

1999-Present Associate Director
Wyeth Vaccines Research

1993-1999 Manager, Molecular Biology and Genetics
Wyeth-Lederle Vaccines and Pediatrics
211 Bailey Road
West Henrietta, New York 14586

1987-1993 Assistant Professor, Department of Microbiology
Georgetown University School of Medicine
3900 Reservoir Rd., N.W.
Washington, DC 20007

1984-1987 Staff Fellow, Office of Biologics
Division of Bacterial Products, FDA
8800 Rockville Pike
Bethesda, MD 20892

1982-1984 Postdoctoral Fellow
Division of Pediatric Infectious Diseases
Johns Hopkins University School of Medicine
Baltimore, MD 21205

EDUCATION:

1978-1982 Graduate School:
Department of Medical Microbiology
Stanford University School of Medicine
Stanford, CA
Ph.D. awarded March 1983

1974-1978 College:
B.A., Luther College, Decorah, Iowa
Majors: Chemistry and Biology

SUMMARY OF EXPERIENCE:

I did my graduate work with Dr. Bruce Stocker at Stanford University, where I developed aromatic-deficient mutants for use as live vaccines (Nature 291:238-239, 1981). I have considerable experience in classical bacterial genetics, Salmonella pathogenicity, and animal models of infection. Through postdoctoral work with Dr. E.R. Moxon at Johns Hopkins, I began studies on the pathogenesis of H. influenzae. Although my primary appointment at Johns Hopkins was in the Division of Pediatric Infectious Diseases, I was also provided space in Dr. Hamilton Smith's laboratory. This arrangement provided a unique opportunity to study bacterial pathogenesis under the guidance of an experienced infectious disease clinician, while also having access to the facilities and expertise of a first-rate molecular biology lab. It was here that I learned many of the basic techniques of molecular biology. From 1984 to 1987, I continued my work on H. influenzae as a Staff Fellow in the Division of Bacterial Products, Office of Biologics, FDA, where in addition to research, I also did some IND work (mainly in the area of live attenuated vaccines). From 1987 to 1993, I was an Assistant Professor of Microbiology at Georgetown University, where in addition to teaching, I also directed my own laboratory.

My work on H. influenzae focused mainly on the genetics of capsule expression. I showed that the genes involved in type b capsule expression are contained within an 18-kb duplication. This arrangement is highly unstable, and rec-dependent recombination between the two copies leads to high-frequency loss of capsule expression. The existing duplication in type b strains can also lead to further amplification of capsule gene sequences, and a significant percentage of invasive isolates possess 3, 4, or even 5 copies of the Cap b repeat. The amplified strains show a gene dosage effect on the amount of capsular polysaccharide produced, and this may provide them with an advantage under certain circumstances. Following licensure of the type b conjugate vaccines, my work shifted toward the use of gene probes as epidemiological markers. These studies focused mainly on nontypable strains, and on the Brazilian Purpuric Fever clone.

In 1993, I moved to what was at the time Lederle-Praxis Biologicals, to manage the Molecular Biology and Genetics Department in the Bacterial Vaccine Discovery program. In addition to providing administrative and scientific oversight for up to 13 scientists, working on eight different bacterial pathogens, I have also served as a scientific project leader, with responsibility for taking a program from Discovery Research into Development. This involved gaining approval of Sr. Management to move the project into the development track, overseeing GMP production of clinical trial materials, and working with Clinical and Regulatory groups on IND filings. I also established and oversee a group within the department to work on optimization of recombinant protein expression.

TEACHING EXPERIENCE:

1988, 1990, 1992	Graduate Course in Microbial Pathogenesis (lecture responsibilities were shared with one other faculty member).
1988, 1990, 1992	Graduate Course in Microbial Genetics (lectures shared with one other faculty member).

1987-1993	Portions of team-taught Medical Microbiology for medical students, including laboratory section.
1980, 1981	Teaching Assistant, Medical School Microbiology Course, Stanford University
1981	Microbiology Laboratory Instructor, California College of Podiatric Medicine, San Francisco,CA
1979	Teaching Assistant, Undergraduate Microbiology Course, Stanford University
1977, 1978	Physiology Laboratory Teaching Assistant, Luther College
1977	Microbiology Laboratory Teaching Assistant, Luther College

FELLOWSHIPS/DISTINCTIONS:

1983-1984	Recipient, NIH Postdoctoral Fellowship
1981 (Summer)	Participant and Scholarship Recipient, Cold Spring Harbor Advanced Bacterial Genetics Course
1979-1982	Recipient, NSF Predoctoral Fellowship
1978-1979	Recipient, NIH Cellular and Molecular Biology Training Grant
1978	Presidential Outstanding Graduate, Luther College
1974	Valedictorian, Paynesville High School, Paynesville, Minnesota

PROFESSIONAL SOCIETIES:

1979-present	Member, American Society for Microbiology
1979	Elected to Sigma Xi

EXTRAMURAL GRANT SUPPORT:

1988-1993	Principal Investigator, "Genetic Analysis of H.influenzae Capsule Expression," NIH, # AI26148; Total Direct Costs: $331,144.

COMMITTEE & ADVISORY WORK:

1988-1991	Member, Georgetown University Animal Care and Use Committee.

1988-1990	Consultant, NIH grant ("Lipooligosaccharides of H. influenzae"), Department of Medicine, Division of Infectious Diseases, SUNY, Buffalo.
1989, 1990	Grant Reviews, Thrasher Fund.
1985	Project Advisory Panel, FDA, Bureau of Foods (Yersinia contract review).
Ad Hoc Manuscript Reviews:	Current Microbiology; Infection and Immunity; Journal of Infectious Diseases; International Journal of Systematic Microbiology; Microbial Pathogenesis; Reviews of Infectious Diseases; Journal of Bacteriology.

INVITED PRESENTATIONS:

November, 2000, Gonococcal Vaccines, 12th International Pathogenic Neisseria Conference, Galveston, Tx.

May, 2000, Meningococcal Vaccines, Wellcome Trust Meningococcal Genomes Meeting, Sanger Center, Hinxton, UK.

September, 1996 Discussion Moderator, 10th International Pathogenic Neisseria Conference, Baltimore, MD.

March, 1992 Seminar, National Institute of Child Health and Human Development, Bethesda, MD.

December, 1991 Seminar, Department of Microbiology, University of Maryland, College Park.

November, 1991 NIH Conference on Emerging Microbes, Sheraton City Center, Washington, DC.

October, 1991 ICAAC Symposium, Chicago, IL.

April, 1991 Seminar, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD.

October, 1990 Third International Symposium on the Epidemiology, Pathogenesis, and Prevention of Haemophilus influenzae Disease, Veldhoven, The Netherlands.

June, 1990 Workshop on the Genetics of Encapsulated Bacteria, Oxford, England.

April, 1989 Course lecture, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD.

May, 1988 Seminar, Department of Medicine, Division of Infectious Diseases, SUNY, Buffalo.

May, 1988 Workshop on Brazilian Purpuric Fever, CDC, Atlanta, GA.

March, 1987 Seminar, National Institute of Child Health and Human Development, Bethesda, MD.

March, 1987 Seminar, American Society for Microbiology Annual Meeting, Atlanta, GA.

May, 1985 Seminar, Molecular Genetics Group, Department of Pediatrics, Oxford University, Oxford, U.K.

June, 1984 Seminar, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD.

March, 1984 Seminar, Center for Vaccine Development, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.

July, 1980 Seminar, Department of Medicine, School of Veterinary Medicine, University of California, Davis.

PUBLICATIONS

1. Hoiseth, S.K. and Meyerchak, R. Physiologic studies on the heart of Amblema peruviana. Proc. Iowa Acad. Sci. 85:70-73, 1978.

2. Hoiseth, S.K. and Stocker, B.A.D. Aromatic-dependent mutants of Salmonella typhimurium are nonvirulent and effective as live vaccines. Nature 291:238-239, 1981.

3. Robertsson, J.A., Lindberg, A.A., Hoiseth, S.K., and Stocker, B.A.D. Salmonella typhimurium infection in calves: Evaluation of protection and survival of virulent S.typhimurium challenge bacteria after immunization with live or inactivated S. typhimurium vaccine. Infect. Immun.41:742-750, 1983.

4. Stocker, B.A.D., Hoiseth, S.K., and Smith, B.P. Aromatic-dependent Salmonella sp. as live vaccine, in mice and calves. Develop. Biol. Standard. 53:47-54, 1983.

5. Smith, B.P., Reina-Guerra, M., Hoiseth, S.K., Stocker, B.A.D., Habasha, F., Johnson, E., and Merritt, F. Safety and efficacy of aromatic-dependent Salmonella typhimurium as live vaccines in calves. Am. J. Vet. Res. 45:59-66, 1984.

6. Smith, B.P., Reina-Guerra, M., Stocker, B.A.D., Hoiseth, S.K., and Johnson, E. Aromatic-dependent Salmonella dublin as parenteral modified live vaccine for calves. Am. J. Vet. Res. 45:2231-2235, 1984.

7. Moxon, E.R., Zwahlen, A., Rubin, L.G., Hoiseth, S.K., and Connelly, C. Pathogenesis of meningitis: Experimental studies on the molecular basis of Haemophilus influenzae infection. Infection 12:S23-S27, 1984.

8. Hoiseth, S.K., and Stocker, B.A.D. Genes aroA and serC of Salmonella typhimurium constitute an operon. J. Bacteriol. 163:355-361, 1985.

9. Hoiseth, S.K., Connelly, C.J., and Moxon, E.R. Genetics of spontaneous, high-frequency loss of b capsule expression in Haemophilus influenzae. Infect. Immun. 49:389-395, 1985.

10. Hoiseth, S.K., Moxon, E.R., and Silver, R.P. Genes involved in Haemophilus influenzae type b capsule expression are part of an 18-kb tandem duplication. Proc. Natl. Acad. Sci. 83:1106-1110, 1986.

11. Moxon, E.R., Ely, S., Kroll, J.S., Allan, I., Zamze, S., Tippett, J., Fulford, S., Hoiseth, S.K. Genetic basis of virulence and type b capsule expression in H. influenzae. In: Lark, D.L., ed. Protein-Carbohydrate Interactions in Biological Systems (FEMS Symposium no. 31). London: Academic Press, 1986; 361-67.

12. Hoiseth, S.K., and Gilsdorf, J.R. 1988. The relationship between type b and nontypable Haemophilus influenzae isolated from the same patient. J. Infect. Dis. 158:643-645.

13. Carlone, G.M., Gorelkin, L., Gheesling, L.L., Hoiseth, S.K., Mulks, M.H., O'Connor, S.P., Weyent, R.S., Myrick, J.E., Mayer, L.W., Arko, R.J. and the Brazilian Purpuric Fever Study Group. Potential virulence factors of Haemophilus influenzae biogroup aegyptius in Brazilian purpuric fever. The Pediatr. Infect. Dis. J. 8:245-247, 1989.

14. Carlone, G.M., Gorelkin, L., Gheesling, L.L., Erwin, A.L., Hoiseth, S.K., Mulks, M.H., O'Connor, S.P., Weyent, R.S., Myrick, J., Rubin, L., Munford, R.S. III, White, E.H., Arko, R.J., Swaminathan, B., Graves, L., Mayer, L.W., Robinson, M.K., Caudill, S.P., and the Brazilian Purpuric Fever Study Group. Potential virulence-associated factors in Brazilian purpuric fever. J. Clin. Microbiol. 27:609-614, 1989.

15. Lagos, R., Avendano, A., Horwitz, I., Musser, J.M., Hoiseth, S.K., Maneval, D.R., Jr., Jones, M.J., Levine, M.M., Dattas, J.P., Prenzel, I., Enriquez, N., Topelberg, S., Olivari, F., and Morris, J.G., Jr. Molecular epidemiology of Haemophilus influenzae within families in Santiago, Chile. J. Infect. Dis. 164:1149-53, 1991.

16. Hoiseth, S.K. The Genus Haemophilus. In: A. Balows, H.G. Truper, M. Dworkin, W. Harder, K.H. Schleifer (ed.), The Prokaryotes, A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Springer-Verlag, New York, NY, 1992; 3304-3330.

17. Hoiseth, S.K., Corn, P.G., and Anders, J. Amplificaton status of capsule genes in Haemophilus influenzae. J. Infect. Dis. 165 (suppl. 1):S114, 1992.

18. Noel, G.J., Hoiseth, S.K., and Edelson, P.J. Type b capsule inhibits ingestion of Haemophilus influenzae by murine macrophages: Studies employing isogenic encapsulated and unencapsulated strains. J. Infect. Dis. 166:178-182, 1992.

19. Corn, P.G., Anders, J., Takala, A.K., Käyhty, H., and Hoiseth, S.K. Genes involved in Haemophilus influenzae type b capsule expression are frequently amplified. J. Infect. Dis. 167:356-364, 1993.

20. Matsuka, Y.V., Dilts, D., Hoiseth, S., and Arumugham, R. Characterization of subunit structure and stability of the recombinant porin from Neisseria gonorrhoeae. J. Protein Chem. 17: 719-728, 1998.

21. Hoiseth, S.K. Vaccines, Bacterial. In: Lederberg, J. (Editor in Chief). (2000). Encyclopedia of Microbiology, Vol. 4, Second edition, 767-778, Academic Press, San Diego .

22. Hoiseth, S.K. Vaccines, Bacterial. In: M. Schaechter (ed.) (2003). Desk Encyclopedia of Microbiology, Elsevier, San Diego.

ABSTRACTS

1. Stocker, B.A.D., and Hoiseth, S.K. Effect of genetic defects in iron assimilation or aromatic biosynthesis on virulence of Salmonella typhimurium. ASM Abst. p.19, 1979.

2. Hoiseth, S.K., and Stocker, B.A.D. Aro$^-$ mutants as live Salmonella vaccines. ASM Abst. p.35, 1982.

3. Mukker, T.K.S., Stocker, B.A.D., Hoiseth, S.K., and Lascelles, A.K. Immunization of mice against homologous and heterologous Salmonella typhimurium infections by use of aromatic-dependent S. typhimurium as live vaccine. Abst. Austral. Soc. Microbiol. 1984.

4. Mukker, T.K.S., McDowell, G.H., Stocker, B.A.D., Hoiseth, S.K., and Lascelles, A.K. Prevention of ovine salmonellosis by immunization with live, aromatic-dependent mutants of Salmonella typhimurium of bovine origin. Abst. Austral. Soc. Microbiol. 1984.

5. Hoiseth, S.K., Connelly, C., and Moxon, E.R. Genetic analysis of high-frequency capsule loss in Haemophilus influenzae type b. ASM Abst. p.67, 1984.

6. Hoiseth, S.K., Moxon, E.R., and Silver, R.P. Genes involved in Haemophilus influenzae type b capsule expression are part of an 18-kb tandem duplication. ICAAC Abst., p.298, 1985.

7. Noel, G.J., Corn, P., and Hoiseth, S. Fluid phase type b capsule (PRP) inhibits phagocytosis of H. influenzae (HI): A potential mechanism for virulence of isolates with multiple copies of cap b genes (CBG). Ped. Res. 29: 180A, 1991.

8. Noel, G.J., Anders, J., Hoiseth, S.K., and Edelson, P.J. A 9-kb EcoRI fragment required for type b capsule expression is associated with resistance of H. influenzae (HI) to phagocytosis by macrophages (MACS). Ped. Res. 29: 181A, 1991.

9. Hoiseth, S.K. Lessons from the molecular biology of H. influenzae: Helping us understand pathogenicity. ICAAC Abst., S-62, p. 368, 1991.